

**What is claimed is:**

1. A mixed sequence oligonucleotide comprising at least 12 nucleotides in length and having a 3' end and a 5' end and divided into a first portion and a further portion,

5 said first portion being capable of supporting cleavage of a complementary target RNA by human RNase H1 polypeptide,

said further portion being incapable of supporting said cleavage by said RNase H1;

10 wherein said first portion comprises at least 6 nucleotides and is positioned in said oligonucleotide such that at least one of said 6 nucleotides is 8 to 12 nucleotides from the 3' end of said oligonucleotide.

2. The oligonucleotide of claim 1 comprising at 15 least one CA nucleotide sequence within said first portion.

3. The oligonucleotide of claim 1 comprising from about 12 to about 50 nucleotides.

4. The oligonucleotide of claim 1 comprising from about 12 to about 25 nucleotides.

20 5. The oligonucleotide of claim 1 wherein each of said nucleotides of said first portion have B-form conformational geometry and are joined together in a continuous sequence.

6. The oligonucleotide of claim 1 wherein each of 25 said nucleotides of said first portion is, independently, a 2'-deoxyribonucleotide, a 2'-SCH<sub>3</sub> ribonucleotide, a 2'-NH<sub>2</sub> ribonucleotide, a 2'-NH(C<sub>1</sub>-C<sub>2</sub> alkyl) ribonucleotide, a 2'-N(C<sub>1</sub>-C<sub>2</sub> alkyl)<sub>2</sub> ribonucleotide, a 2'-CF<sub>3</sub> ribonucleotide, a

2'= $\text{CH}_2$  ribonucleotide, a 2'= $\text{CHF}$  ribonucleotide, a 2'= $\text{CF}_3$  ribonucleotide, a 2'- $\text{CH}_3$  ribonucleotide, a 2'- $\text{C}_2\text{H}_5$  ribonucleotide, a 2'- $\text{CH}=\text{CH}_2$  ribonucleotide or a 2'- $\text{C}\equiv\text{CH}$  ribonucleotide.

5       7. The oligonucleotide of claim 1 wherein each of said nucleotides of said first portion is a 2'-deoxyribonucleotide.

10      8. The oligonucleotide of claim 1 wherein each of said nucleotide of said first portion is, independently, a 2'-CN arabinonucleotide, a 2'-F arabinonucleotide, a 2'-Cl arabinonucleotide, a 2'-Br arabinonucleotide, a 2'-N<sub>3</sub> arabinonucleotide, a 2'-OH arabinonucleotide, a 2'-O-CH<sub>3</sub> arabinonucleotide or a 2'-dehydro-2'-CH<sub>3</sub> arabinonucleotide.

15      9. The oligonucleotide of claim 1 wherein each of said nucleotides of said first portion is, independently, a 2'-F arabinonucleotide, a 2'-OH arabinonucleotide or a 2'-O-CH<sub>3</sub> arabinonucleotide.

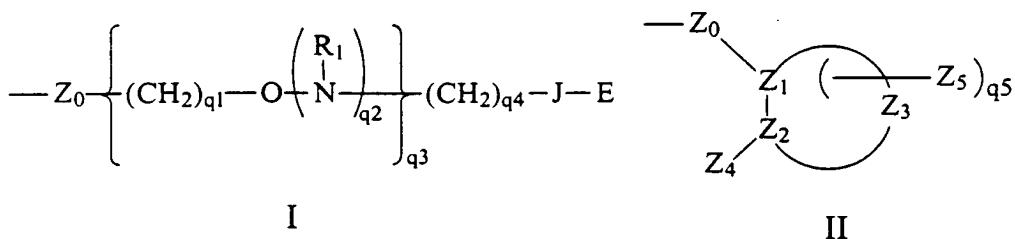
20      10. The oligonucleotide of claim 1 wherein each of said nucleotides of said first portion is, independently, a 2'-F arabinonucleotide or a 2'-OH arabinonucleotide.

11. The oligonucleotide of claim 1 wherein said nucleotides of said first portion are joined together in said continuous sequence by phosphate, phosphorothioate, phosphorodithioate or boranophosphate linkages.

25      12. The oligonucleotide of claim 1 wherein said further portion includes a plurality of nucleotides, at least some of said nucleotides comprise a 2' substituent group wherein each substituent group is, independently,

hydroxyl, C<sub>1</sub>-C<sub>20</sub> alkyl, C<sub>2</sub>-C<sub>20</sub> alkenyl, C<sub>2</sub>-C<sub>20</sub> alkynyl, halogen, amino, thiol, keto, carboxyl, nitro, nitroso, nitrile, trifluoromethyl, trifluoromethoxy, O-alkyl, O-alkenyl, O-alkynyl, S-alkyl, S-alkenyl, S-alkynyl, NH-alkyl, NH-alkenyl, NH-alkynyl, N-dialkyl, O-aryl, S-aryl, NH-aryl, O-aralkyl, S-aralkyl, NH-aralkyl, N-phthalimido, imidazole, azido, hydrazino, hydroxylamino, isocyanato, sulfoxide, sulfone, sulfide, disulfide, silyl, aryl, heterocycle, carbocycle, intercalator, reporter molecule, conjugate, polyamine, polyamide, polyalkylene glycol, or polyether;

or each substituent group has one of formula I or II:

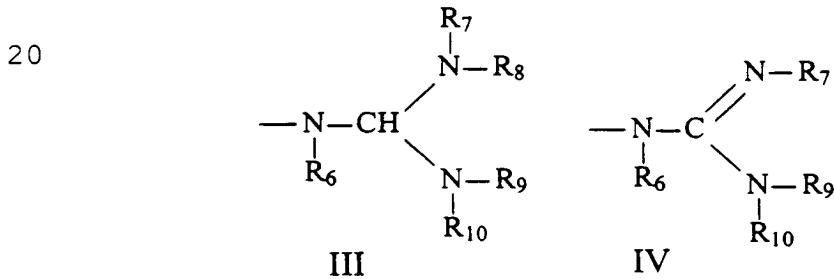


wherein:

Z<sub>0</sub> is O, S or NH;

J is a single bond, O or C(=O);

E is C<sub>1</sub>-C<sub>10</sub> alkyl, N(R<sub>1</sub>)(R<sub>2</sub>), N(R<sub>1</sub>)(R<sub>5</sub>), N=C(R<sub>1</sub>)(R<sub>2</sub>), N=C(R<sub>1</sub>)(R<sub>5</sub>) or has one of formula III or IV;



each R<sub>6</sub>, R<sub>7</sub>, R<sub>8</sub>, R<sub>9</sub> and R<sub>10</sub> is, independently, hydrogen, C(O)R<sub>11</sub>, substituted or unsubstituted C<sub>1</sub>-C<sub>10</sub> alkyl, substituted or unsubstituted C<sub>2</sub>-C<sub>10</sub> alkenyl, substituted or unsubstituted C<sub>2</sub>-C<sub>10</sub> alkynyl, alkylsulfonyl, arylsulfonyl, a

chemical functional group or a conjugate group, wherein the substituent groups are selected from hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl and alkynyl;

5       or optionally, R<sub>7</sub> and R<sub>8</sub>, together form a phthalimido moiety with the nitrogen atom to which they are attached;

          or optionally, R<sub>9</sub> and R<sub>10</sub>, together form a phthalimido moiety with the nitrogen atom to which they are attached;

          each R<sub>11</sub> is, independently, substituted or

10      unsubstituted C<sub>1</sub>-C<sub>10</sub> alkyl, trifluoromethyl, cyanoethoxy, methoxy, ethoxy, t-butoxy, allyloxy, 9-fluorenylmethoxy, 2-(trimethylsilyl)-ethoxy, 2,2,2-trichloroethoxy, benzyloxy, butyryl, iso-butyryl, phenyl or aryl;

          R<sub>5</sub> is T-L,

15      T is a bond or a linking moiety;

          L is a chemical functional group, a conjugate group or a solid support material;

          each R<sub>1</sub> and R<sub>2</sub> is, independently, H, a nitrogen protecting group, substituted or unsubstituted C<sub>1</sub>-C<sub>10</sub> alkyl,

20      substituted or unsubstituted C<sub>2</sub>-C<sub>10</sub> alkenyl, substituted or unsubstituted C<sub>2</sub>-C<sub>10</sub> alkynyl, wherein said substitution is OR<sub>3</sub>, SR<sub>3</sub>, NH<sub>3</sub><sup>+</sup>, N(R<sub>3</sub>)(R<sub>4</sub>), guanidino or acyl where said acyl is an acid amide or an ester;

          or R<sub>1</sub> and R<sub>2</sub>, together, are a nitrogen protecting group

25      or are joined in a ring structure that optionally includes an additional heteroatom selected from N and O;

          or R<sub>1</sub>, T and L, together, are a chemical functional group;

          each R<sub>3</sub> and R<sub>4</sub> is, independently, H, C<sub>1</sub>-C<sub>10</sub> alkyl, a

30      nitrogen protecting group, or R<sub>3</sub> and R<sub>4</sub>, together, are a nitrogen protecting group;

          or R<sub>3</sub> and R<sub>4</sub> are joined in a ring structure that optionally includes an additional heteroatom selected from N and O;

$Z_4$  is  $OX$ ,  $SX$ , or  $N(X)_2$ ;

each  $X$  is, independently,  $H$ ,  $C_1-C_8$  alkyl,  $C_1-C_6$  haloalkyl,  $C(=NH)N(H)R_5$ ,  $C(=O)N(H)R_5$  or  $OC(=O)N(H)R_5$ ;

$R_5$  is  $H$  or  $C_1-C_8$  alkyl;

5  $Z_1$ ,  $Z_2$  and  $Z_3$  comprise a ring system having from about 4 to about 7 carbon atoms or having from about 3 to about 6 carbon atoms and 1 or 2 hetero atoms wherein said hetero atoms are selected from oxygen, nitrogen and sulfur and wherein said ring system is aliphatic, unsaturated  
10 aliphatic, aromatic, or saturated or unsaturated heterocyclic;

$Z_5$  is alkyl or haloalkyl having 1 to about 10 carbon atoms, alkenyl having 2 to about 10 carbon atoms, alkynyl having 2 to about 10 carbon atoms, aryl having 6 to about  
15 14 carbon atoms,  $N(R_1)(R_2)$  OR<sub>1</sub>, halo, SR<sub>1</sub> or CN;

each  $q_1$  is, independently, an integer from 1 to 10;

each  $q_2$  is, independently, 0 or 1;

$q_3$  is 0 or an integer from 1 to 10;

$q_4$  is an integer from 1 to 10; and

20  $q_5$  is from 0, 1 or 2;

provided that when  $q_3$  is 0,  $q_4$  is greater than 1.

13. The oligonucleotide of claim 1 wherein each of said nucleotides of said further portion is, independently, a 2'-F ribonucleotide, a 2'-O-( $C_1-C_6$  alkyl) ribonucleotide,  
25 or a 2'-O-( $C_1-C_6$  substituted alkyl) ribonucleotide wherein the substitution is  $C_1-C_6$  ether,  $C_1-C_6$  thioether, amino, amino( $C_1-C_6$  alkyl) or amino( $C_1-C_6$  alkyl)<sub>2</sub>.

14. The oligonucleotide of claim 1 wherein said  
30 nucleotides of said further portion are joined together in a continuous sequence by 3'-5' phosphodiester, 2'-5' phosphodiester, phosphorothioate, Sp phosphorothioate, Rp phosphorothioate, phosphorodithioate, 3'-deoxy-3'-amino

phosphoroamidate, 3'-methylenephosphonate, methylene(methylimino), dimethylhydrazino, amide 3, amide 4 or boranophosphate linkages.

15. The oligonucleotide of claim 1 wherein at least  
5 two of said nucleotides of said further portion are joined  
together in a continuous sequence that is positioned 3' to  
said first portion.

16. The oligonucleotide of claim 1 wherein at least  
two of said nucleotides of said further portion are joined  
10 together in a continuous sequence that is positioned 5' to  
said first portion.

17. The oligonucleotide of claim 1 wherein at least  
two of said nucleotides of said further portion are joined  
together in a continuous sequence that is positioned 3' to  
15 said first portion and at least two of said further portion  
are joined together in a continuous sequence that is  
positioned 5' to said first portion.

18. The oligonucleotide of claim 1 wherein at least  
four of said nucleotides of said further portion are joined  
20 together in a continuous sequence that is positioned 3' to  
said first portion.

19. The oligonucleotide of claim 1 wherein at least  
four of said nucleotides of said further portion are joined  
together in a continuous sequence that is positioned 5' to  
25 said first portion.

20. The oligonucleotide of claim 1 wherein at least  
four of said nucleotides of said further portion are joined  
together in a continuous sequence that is positioned 3' to

said first portion and at least four of said nucleotides of said further portion are joined together in a continuous sequence that is positioned 5' to said first portion.

21. A mixed sequence oligonucleotide comprising at 5 least 8 nucleotides and having a CA nucleotide sequence wherein at least one of the two nucleotides of said CA sequence is positioned 8 to 12 nucleotides from the 3' end of said oligonucleotide.

22. The oligonucleotide of claim 21 wherein said 10 oligonucleotide is capable of supporting cleavage of a complementary target RNA by human RNase H1 polypeptide.

23. A mixed sequence chimeric oligonucleotide comprising at least 8 nucleotides and having a CA nucleotide sequence wherein at least one of the two 15 nucleotides of said CA sequence is positioned 8 to 12 nucleotides from the 3' end of said oligonucleotide.

24. The chimeric oligonucleotide of claim 23 wherein said oligonucleotide is capable of supporting cleavage of a complementary target RNA by human RNase H1 polypeptide.

20 25. A mixed sequence oligonucleotide comprising 8 to 25 nucleotides and having a CA nucleotide sequence wherein at least one of the nucleotides of said CA sequence is positioned 8 to 12 nucleotides from the 3' end of said oligonucleotide.

25 26. A mixed sequence chimeric oligonucleotide comprising 8 to 25 nucleotides and having a CA nucleotide sequence wherein at least one of the nucleotides of said CA sequence is positioned 8 to 12 nucleotides from the 3' end

of said oligonucleotide.

27. A chimeric oligonucleotide comprising 8 to 25 nucleotides and having a portion capable of supporting cleavage of a complementary target RNA by human RNase H1 5 polypeptide wherein said portion supporting said cleavage is at least 6 nucleotides in length and is positioned in said oligonucleotide such that at least one of said 6 nucleotides is positioned 8 to 12 nucleotides from the 3' end of said oligonucleotide.

10 28. The oligonucleotide of claim 27 wherein said oligonucleotide comprises at least one CA nucleotide sequence within said portion supporting said cleavage.